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Neutrophil Gelatinase-Associated Lipocalin for Acute Kidney Injury During Acute Heart Failure Hospitalizations

The AKINESIS Study

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ABSTRACT

BACKGROUND Worsening renal function (WRF) often occurs during acute heart failure (AHF) and can portend adverse outcomes; therefore, early identification may help mitigate risk. Neutrophil gelatinase-associated lipocalin (NGAL) is a novel renal biomarker that may predict WRF in certain disorders, but its value in AHF is unknown.

OBJECTIVES This study sought to determine whether NGAL is superior to creatinine for prediction and/or prognosis of WRF in hospitalized patients with AHF treated with intravenous diuretic agents.

METHODS This was a multicenter, prospective cohort study enrolling patients presenting with AHF requiring intravenous diuretic agents. The primary outcome was whether plasma NGAL could predict the development of WRF, defined as a sustained increase in plasma creatinine of 0.5 mg/dl or ≥50% above first value or initiation of acute renal-replacement therapy, within the first 5 days of hospitalization. The main secondary outcome was in-hospital adverse events.

RESULTS We enrolled 927 subjects (mean age, 68.5 years; 62% men). The primary outcome occurred in 72 subjects (7.8%). Peak NGAL was more predictive than the first NGAL, but neither added significant diagnostic utility over the first creatinine (areas under the curve: 0.656, 0.647, and 0.652, respectively). There were 235 adverse events in 144 subjects. The first NGAL was a better predictor than peak NGAL, but similar to the first creatinine (areas under the curve: 0.691, 0.653, and 0.686, respectively). In a post hoc analysis of subjects with an estimated glomerular filtration rate <60 ml/min/1.73 m^2, a first NGAL <150 ng/ml indicated a low likelihood of adverse events.

CONCLUSIONS Plasma NGAL was not superior to creatinine for the prediction of WRF or adverse in-hospital outcomes. The use of plasma NGAL to diagnose acute kidney injury in AHF cannot be recommended at this time. (Acute Kidney Injury Neutrophil Gelatinase-Associated Lipocalin [N-GAL] Evaluation of Symptomatic Heart Failure Study [AKINESIS]; NCT01291836) (J Am Coll Cardiol 2016;68:1420–31) © 2016 by the American College of Cardiology Foundation.

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Heart failure (HF) is a major global health problem, with more than 23 million people afflicted worldwide (1). Kidney dysfunction is common among patients hospitalized with acute HF (AHF) and portends a worse prognosis (2–4). A strong interaction is recognized between the heart and kidney, and worsening renal function (WRF) can occur with treatment of AHF with adverse outcomes (5,6). Creatinine is currently the standard biomarker for renal function; however, it has a delayed increase after kidney injury. Furthermore, WRF in AHF, reflected by a rise in creatinine, may not reflect acute kidney injury (AKI) and may not be prognostic in all patients (7). Novel biomarkers are needed for earlier detection of WRF that is associated with adverse outcomes (8).

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Neutrophil gelatinase-associated lipocalin (NGAL) is a small molecule of the lipocalin family of proteins. Found on the brush-border of renal tubular cells, NGAL increases during the acute phase of toxic and ischemic kidney injury (9). Plasma NGAL has predictive value for AKI in multiple conditions, including cardiac surgery, contrast-induced nephropathy, and critical illness (10–12). It is also elevated in patients with chronic HF and is associated with adverse outcomes, but its value in patients with AHF is not well established (13,14). Single-center studies have suggested that NGAL predicts WRF in AHF; however, it is unknown if this can be reproduced on a larger scale, or if NGAL is specifically useful with intravenous (IV) diuretic therapy (15–17).

AKINESIS (Acute Kidney Injury N-gal Evaluation of Symptomatic heart faIlure Study) is an international, multicenter, prospective cohort study enrolling patients presenting with AHF. A primary goal was to examine plasma NGAL's ability to predict WRF or need for renal-replacement therapy (RRT) in patients with AHF treated with diuretic agents. A secondary goal was to assess prognostic ability for in-hospital adverse outcomes.

METHODS

STUDY DESIGN. AKINESIS was an international, multicenter, prospective cohort study enrolling patients presenting to the emergency department or hospital with signs and symptoms of AHF, with planned admission and treatment with diuretic agents. Abbott Laboratories and Alere, Inc., jointly sponsored the study. Subjects were enrolled at 16 sites, 7 in the United States and 9 in Europe, and followed to discharge (NCT01291836).

The principal investigators and sponsors designed and oversaw the trial. Each center's institutional review board approved the study. All participants provided written informed consent. Data were collated at a core data management facility. The principal investigators had full access to the database. An independent biostatistician performed statistical analysis. The initial manuscript was written by the first 2 authors, and reviewed and edited by all authors. All authors vouch for the accuracy of the reported findings.

PARTICIPANTS. Subjects at least 18 years of age, presenting to the emergency department or hospital with AHF, were screened for inclusion and enrolled as early as possible. Subjects had to have 1 or more signs or symptoms of HF, including dyspnea on exertion, rales or crackles, galloping heart rhythm, jugular venous distention, orthopnea, paroxysmal nocturnal dyspnea, using more than 2 pillows to sleep, fatigue, edema, frequent coughing, a cough that produces mucous or blood-tinged sputum, or a dry cough when lying flat. In addition, subjects must have received or planned treatment with IV diuretic agents. Subjects had to be able to comply with all aspects of the protocol and give consent.

ABBREVIATIONS AND ACRONYMS

AHF = acute heart failure
AKI = acute kidney injury
AUC = area under the curve
BUN = blood urea nitrogen
CI = confidence interval
eGFR = estimated glomerular filtration rate
HF = heart failure
IV = intravenous
NGAL = neutrophil gelatinase-associated lipocalin
RRT = renal-replacement therapy
WRF = worsening renal function

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Subjects were excluded if: 1) symptoms consistent with acute coronary syndrome were the chief cause of the current AHF episode; 2) they were already on dialysis before enrollment or if dialysis initiation was planned during the current hospitalization; 3) they had a heart, lung, kidney, or liver transplant; 4) they had participated in a drug treatment study within the past 30 days or had already enrolled in this study; or 5) they were pregnant or belonging to an institutional review board-determined vulnerable population.

ENDPOINTS. The primary outcome was whether plasma NGAL predicted the development of either WRF, reflected by a sustained increase in creatinine of ≥0.5 mg/dl (44.2 μmol/l) or ≥50% of the first creatinine value obtained, or the initiation of acute RRT within 5 days of being hospitalized. A sustained increase was defined as a creatinine value meeting criteria on at least 2 consecutive days. Acute RRT modalities included dialysis, ultrafiltration, and hemofiltration. The predictive ability of NGAL for each endpoint of WRF and RRT was also tested.

The major secondary endpoint was the ability of NGAL to predict adverse in-hospital outcomes. These included severe WRF (a sustained increase ≥100% of the first creatinine value); initiation of RRT; death; nephrology consultation; and admission to the intensive care unit with need for mechanical ventilation, inotropic/vasopressor support, or both.

Pre-specified secondary endpoints included whether an elevated NGAL on admission predicted which subjects with an admission estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 would have a greater decrease in eGFR at discharge. Pre-specified alternative definitions of WRF included a sustained increase in creatinine ≥0.3 mg/dl (26.5 μmol/l) or ≥50% of the first creatinine, and a sustained increase in creatinine ≥50% of the first creatinine, both during the first 5 days of hospitalization. Although the latter definition is similar to RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) criteria, the KDIGO (Kidney Disease: Improving Global Outcomes) criteria for AKI, which are similar to AKIN (Acute Kidney Injury Network) criteria, were published during study enrollment. This definition did not use a sustained increase in creatinine; thus, post hoc analyses without sustained increases were performed. These included a nonsustained increase of ≥0.5 mg/dl or ≥50% of the first creatinine and a nonsustained increase in creatinine of ≥0.3 mg/dl or ≥50% of the first creatinine, both during the first 5 days of hospitalization, and any nonsustained increase of ≥0.3 mg/dl or ≥50% of the first creatinine at any time during the hospitalization.

Another post hoc analysis, prompted by an evolution in published reports on the evaluation of novel renal biomarkers during the study, tested the predictive value of NGAL stratified by admission eGFR and first NGAL for the combined endpoint of in-hospital adverse outcomes (8). eGFR was stratified at 60 ml/min/1.73 m^2. NGAL was assessed using 2 cutpoints: the 80% sensitivity cutpoint, on the basis of our analysis, and at 150 ng/dl, on the basis of a prior study (11).

The primary predictive metric was the peak NGAL value. The predictive value of the first NGAL, first creatinine, and the combination of the first creatinine and first NGAL were also analyzed. Peak NGAL was defined as the highest value obtained at least 6 h before the event, if the event time was available, or at least a day before the event, if only the event date was available. If the event occurred on the first day, or the only available NGAL value was within 6 h of the event, this was used. If an NGAL value was only available after the event occurred, this was not used. This peak value for subjects with an outcome was compared with the peak value from all collected samples in subjects without the outcome. The first NGAL was the value obtained only at the first collection time. The first creatinine was the first value obtained.

DATA COLLECTION. After subjects were screened and gave written consent, demographics, vital signs, HF signs and symptoms on presentation, medications, and past medical history were collected. Laboratory samples were collected as per standard of care determined by the treating physician, and were analyzed at the clinical laboratories of the treating hospital. If collected, values recorded on admission and discharge included white blood cell count, hemoglobin, hematocrit, sodium, potassium, blood urea nitrogen (BUN), creatinine, alanine aminotransferase, aspartate aminotransferase, troponin, and natriuretic peptides.

During the hospitalization, sodium, potassium, BUN, and creatinine values were recorded for at least the first 7 days of hospitalization. These were obtained at the discretion of the treating physician. In-hospital adverse outcomes listed previously were recorded.

NGAL ASSESSMENT. Specimens for NGAL assessment were collected at 6 time points if the subject remained hospitalized during the collection period. The first specimen was collected the day of enrollment, within 2 h of the first IV diuretic dose. The second specimen was collected 2 to 6 h later.

The third, fourth, and fifth specimens were collected on hospital day 1, 2, and 3, respectively. The sixth specimen was collected on the day of discharge or anticipated discharge. Compliance with sample collection was high, except for the discharge sample, with 98.3%, 92.0%, 94.7%, 96.7%, 94.5%, and 65.4% of samples collected at the listed time points. Plasma NGAL specimens were frozen and shipped to the core laboratory for analysis with the Alere Triage plasma NGAL assay.

STATISTICAL ANALYSES. Assuming an event rate of 9% and an area under the curve (AUC) of 0.70 for NGAL for the primary outcome, it was estimated that 60 primary events had to occur to have 80% power to detect a difference of 0.10 in AUC. With a 20% margin of error, anticipated enrollment was 800 subjects; however, as enrollment neared 800, the event rate was lower than anticipated. It was decided to continue enrollment until at least 60 events had occurred, resulting in 930 subjects enrolled. The institutional review boards were notified of the planned increase and no site exceeded its enrollment limit.

Baseline variables are presented as means and standard deviations for normally distributed continuous variables, medians with interquartile ranges for nonnormalized variables, and percentages for categorical variables. When the status of a comorbidity was unknown, it was assumed to not be present.

Baseline characteristics between subjects with and without the primary outcome were compared using the Student *t* test, chi-square test, and Mann-Whitney *U* test, as appropriate. To explore the independent predictive value of NGAL and potential influence of covariates on the primary outcome, univariable logistic regression analysis was used. Variables with a p value <0.10 were retained for forward, stepwise, multivariate logistic regression analyses, and those with a p value <0.05 were retained for a multivariate model. The log-transformed values of peak and first NGAL were added separately to the model, and the C statistic was calculated with and without NGAL. When NGAL and creatinine were assessed in combination, logistic regression was used and predictors were log-transformed. Receiver operating characteristic curves were generated to determine the AUC with 95% confidence intervals (CIs) for peak NGAL, first NGAL, first creatinine, and combined first NGAL and first creatinine for the primary and secondary outcomes. Cutpoints for 80% sensitivity and 80% specificity were determined. Kaplan-Meier curves were created for time to events of the primary and combined secondary endpoint. Spearman correlation was used to assess change in subjects with eGFR <60 ml/min/1.73 m^2 and NGAL values. Subgroups were compared by chi-square test, and sensitivity, specificity, and positive and negative predictive values in the specific subgroups were calculated. A p value <0.05 was considered significant. All statistical calculations were performed on SPSS version 19 (SPSS, Inc., Chicago, Illinois).

RESULTS

PATIENT DEMOGRAPHICS. From January 2011 through September 2013, a total of 930 subjects were enrolled. One subject was later found not to meet inclusion criteria, 1 met exclusion criteria, and 1 withdrew consent, leaving 927 subjects for analysis. All subjects had follow-up throughout hospitalization. Subjects were hospitalized for a median of 5 days (interquartile range: 6 days).

The mean age of subjects was 68.5 years, with 62% men and 66.9% identified as white (Table 1). Most subjects had hypertension (80.7%), a significant portion had coronary artery disease (46.1%) and diabetes mellitus (43.6%), and almost 26% had chronic kidney disease. The median admission creatinine was 1.19 mg/dl, with a median eGFR of 57 ml/min/1.73 m^2. The median admission B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide values were 795 pg/dl and 3,446 pg/dl, respectively. Approximately 70% of subjects were on diuretic agents before admission, 70% were on beta-blockers, and almost 63% were on an angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker.

PRIMARY OUTCOME. Seventy-two subjects (7.8%) had the primary outcome (Figure 1A), with 66 (7.1%) subjects having WRF and 11 (1.2%) requiring RRT. Subjects with and without the primary outcome were similar, except that subjects with the primary outcome had a higher prevalence of diabetes and chronic kidney disease (Table 1). The latter was reflected by significantly higher admission creatinine, lower eGFR, and higher BUN. Hemoglobin was also significantly lower. Troponin T was significantly higher in subjects with the primary outcome, whereas troponin I was not different.

A total of 918 subjects had peak NGAL values (9 subjects did not have any samples for NGAL analysis), and 911 had first NGAL values (16 lacked a sample from the first collection time). Peak values occurred a mean of 1.6 ± 1.1 days before the primary outcome, with most events occurring 24 h after admission (Figure 1A). Distributions of peak NGAL and first NGAL

TABLE 1 Baseline Characteristics of Study Patients and Comparison of Those With and Without Primary Outcome

	N	Total	Primary Outcome	No Primary Outcome
Age, yrs	921	68.5 (54.7–82.3)	70.75 (57.1–84.4)	68.3 (54.4–82.1)
Male	927	62.0	52.8	62.8
White	921	66.9	65.3	67.0
Systolic blood pressure, mm Hg	927	139.9 (110.8–169)	145.5 (115.8–175.2)	139.4 (110.4–168.3)
Diastolic blood pressure, mm Hg	927	80.1 (60.8–99.4)	82.1 (59.7–104.5)	79.9 (60.9–99)
Heart rate, beats/min	927	88 (65.2–110.8)	87 (68.3–105.7)	88 (64.9–111.2)
Past medical history				
Acute myocardial infarction	927	27.5	29.2	27.4
Coronary artery disease	927	46.1	54.2	45.4
Prior PCI	927	22.5	26.4	22.2
Prior CABG	927	17.0	25.0	16.4
Arrhythmia	927	47.7	44.4	48.0
Hypertension	927	80.7	87.5	80.1
Hyperlipidemia	927	52.4	52.8	52.4
Diabetes*	927	43.6	58.3	42.3
Cerebrovascular accident	927	13.9	8.3	14.4
Peripheral arterial disease	927	3.2	2.8	3.3
COPD	927	26.1	23.6	26.3
Chronic kidney disease†	927	25.9	45.8	24.2
Anemia	927	22.4	29.2	21.9
Liver failure	927	2.8	5.6	2.6
Tobacco use	927	16.6	12.5	17.0
Cancer	927	14.1	13.9	14.2
Prior medications				
Beta-blockers	927	70.2	73.6	69.9
ACE inhibitors	927	43.7	38.9	44.1
Angiotensin-receptor blockers	927	18.9	26.4	18.2
Diuretic agents	927	70.7	68.1	70.9
Antiarrhythmic agent	927	15.3	18.1	15.1
Digoxin	927	11.0	6.9	11.3
Laboratory studies				
Sodium, mg/dl	921	139 [136–141]	139 [136–142]	139 [136–141]
Creatinine, mg/dl†	927	1.19 [0.94–1.6]	1.60 [1.02–2.46]	1.17 [0.93–1.55]
eGFR, ml/min/1.73 m^2†	927	57.0 [40.4–77.8]	38.1 [24.8–66.1]	58.1 [42.4–79.2]
Hemoglobin, g/dl†	924	11.8 [10.1–13.2]	10.4 [8.7–12.4]	11.9 [10.3–13.3]
BUN, mg/dl†	918	23.5 [16.5–36.0]	33.7 [21.3–60.8]	23 [16–35]
BNP, ng/l	529	795 [337–1,486]	810 [501–1,915]	793 [328–1,479]
NT-proBNP, ng/l	316	3,446 [1,552–7,376]	4,814 [2,389–7,567]	3,221 [1,507–7,360]
Troponin T, ng/ml*	343	0.029 [0.015–0.056]	0.045 [0.024–0.080]	0.027 [0.014–0.051]
Troponin I, ng/ml	479	0.04 [0.02–0.07]	0.04 [0.025–0.115]	0.04 [0.02–0.066]

Values are mean (range), %, or median [interquartile range]. *$p < 0.01$. †$p < 0.001$.

ACE = angiotensin-converting enzyme; AKI = acute kidney injury; BNP = B-type natriuretic peptide; BUN = blood urea nitrogen; CABG = coronary artery bypass graft; COPD = chronic obstructive pulmonary disease; eGFR = estimated glomerular filtration rate; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCI = percutaneous coronary intervention.

values for subjects with and without the primary outcome are shown in **Figure 1B**.

For the primary outcome, the AUC, 80% sensitivity cutpoint, and 80% specificity cutpoint for peak NGAL were 0.656 (95% CI: 0.589 to 0.723), 141.0 ng/dl, and 350.0 ng/dl, respectively (**Figure 2A**). The AUC, 80% sensitivity cutpoint, and 80% specificity cutpoint for first NGAL were 0.647 (95% CI: 0.579 to 0.715), 104.4 ng/dl, and 274.9 ng/dl, respectively. In comparison, the AUC for the first creatinine value was 0.652 (95% CI: 0.576 to 0.729). The combination of first creatinine and first NGAL did not enhance predictive ability (AUC: 0.66; 95% CI: 0.586 to 0.733).

In multivariate logistic regression analysis, admission systolic blood pressure, pre-existing chronic kidney disease, admission hemoglobin, and admission BUN retained significance in the model. No interaction was found by region (United States vs. Europe). Peak NGAL ($p = 0.038$) was significant when

added to the multivariate model, but the first NGAL ($p = 0.071$) was not. Findings did not differ on the basis of region. The addition of peak NGAL did not significantly improve the C statistic of the model (AUC increased from 0.707 to 0.711; $p = 0.70$) for the primary outcome.

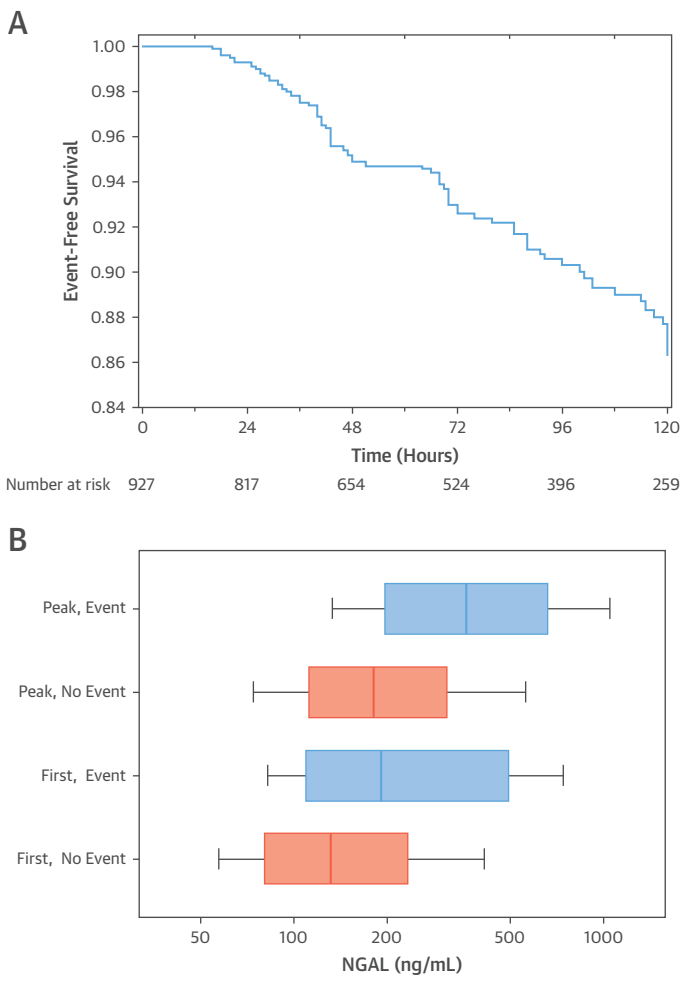
For the individual outcome of WRF, the AUCs for peak NGAL, first NGAL, first creatinine, and combined first NGAL and creatinine were 0.637 (95% CI: 0.566 to 0.708), 0.625 (95% CI: 0.554 to 0.695), 0.631 (95% CI: 0.552 to 0.711), and 0.636 (95% CI: 0.559 to 0.713), respectively. For the individual outcome of RRT, the AUCs for peak NGAL, first NGAL, first creatinine, and combined first NGAL and creatinine were 0.884 (95% CI: 0.796 to 0.961), 0.902 (95% CI: 0.827 to 0.976), 0.876 (95% CI: 0.754 to 0.998), and 0.914 (95% CI: 0.827 to 1.00), respectively.

SECONDARY OUTCOMES. A total of 235 adverse outcomes occurred in 144 subjects (Figure 3A). The distribution of peak NGAL value for subjects with each adverse outcome and those without adverse outcomes are shown in Figure 3B. The most common adverse outcome was nephrology consultation ($n = 59$). A significant number of subjects were admitted to the intensive care unit requiring inotropic/vasopressor support ($n = 51$) or ventilator support ($n = 43$). Twenty-nine subjects (3.1%) died during hospitalization.

The AUC, 80% sensitivity cutpoint, and 80% specificity cutpoint for peak NGAL for the composite secondary outcome were 0.653 (95% CI: 0.601 to 0.704), 124.6 ng/dl, and 331.6 ng/dl, respectively (Figure 3C). AUC, 80% sensitivity cutpoint, and 80% specificity cutpoint for the first NGAL were 0.691 (95% CI: 0.643 to 0.740), 109.3 ng/dl, and 247.5 ng/dl, respectively. AUC for the first creatinine value was 0.686 (95% CI: 0.634 to 0.738). The combination of first creatinine and first NGAL had an AUC of 0.716 (95% CI: 0.666 to 0.766). Because nephrology consultation was the most frequent adverse outcome, an analysis without this outcome was performed. AUCs for peak NGAL, first NGAL, and first creatinine notably decreased, and were 0.595 (95% CI: 0.534 to 0.656), 0.645 (95% CI: 0.589 to 0.701), and 0.623 (95% CI: 0.562 to 0.684), respectively.

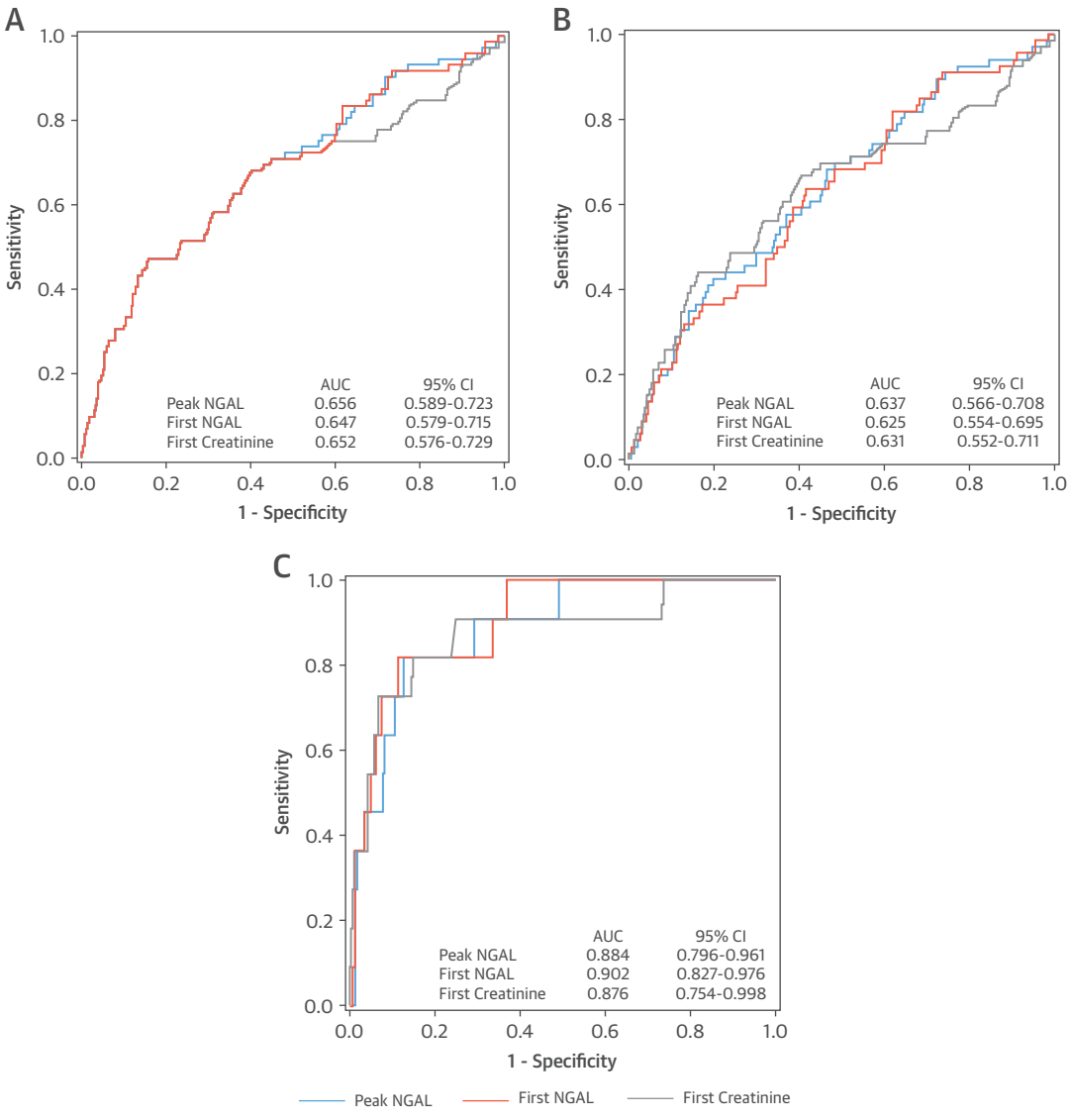
The alternative definitions of WRF evaluated included a sustained increase of creatinine ≥50% of the first creatinine value, a nonsustained increase of creatinine of ≥0.5 mg/dl or ≥50% of the first creatinine value, a sustained increase of creatinine of ≥0.3 mg/dl or ≥50% of the first creatinine value, and a nonsustained increase of creatinine of ≥0.3 mg/dl or ≥50% of the first creatinine value, occurring in the first 5 days, and a nonsustained increase of creatinine of ≥0.3 mg/dl or ≥50% of the first creatinine value at any time. For these definitions, there were 38 (4.1%), 112 (12.1%), 128 (13.8%), 222 (23.9%), and 284 (30.6%) events, respectively. Using the first NGAL, AUCs ranged from 0.488 to 0.625, with the highest AUC for a nonsustained increase in creatinine of ≥0.3 mg/dl or ≥50% of the first creatinine value at any time. Evaluating peak NGAL value only in subjects whose creatinine rose by ≥0.3 mg/dl, AUCs ranged from 0.593 to 0.606. Again, the highest AUC was in those with a nonsustained increase in creatinine of ≥0.3 mg/dl or ≥50% of the first creatinine value at any time. In subjects with an eGFR <60 ml/min/1.73 m^2, there was no correlation between first NGAL and percent decline in eGFR at discharge.

FIGURE 1 Primary Outcome Events and NGAL Values



(A) Kaplan-Meier curve with subjects at risk at the **bottom**. Approximately 86% of subjects were free from the primary outcome at 5 days. **(B)** Peak and first NGAL values are displayed in those with and without the primary outcome. NGAL values were log-transformed for nonnormalized distribution. NGAL = neutrophil gelatinase-associated lipocalin.

FIGURE 2 Receiver Operating Characteristic Curves for the Primary Outcome and Components

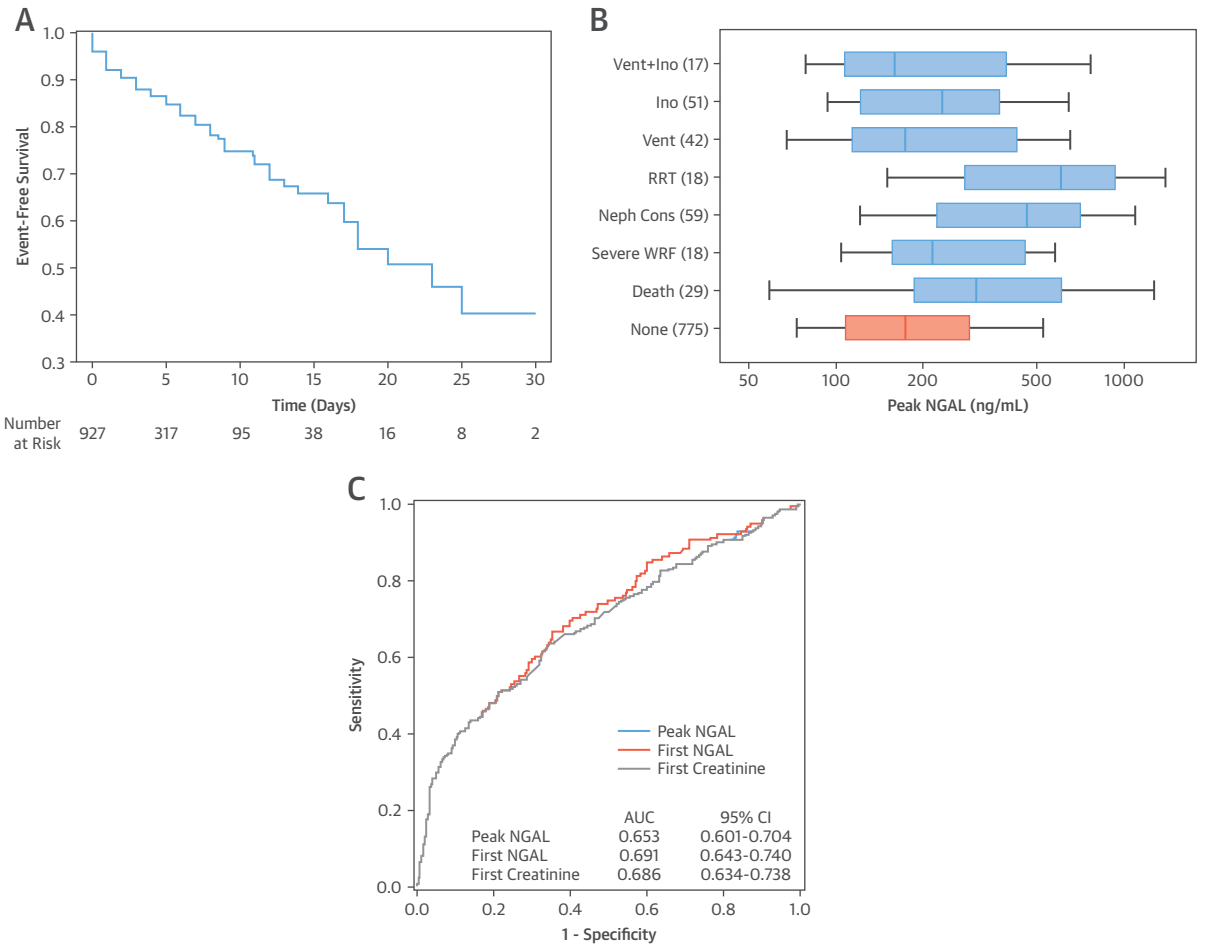


Receiver operating characteristic curves for primary outcome **(A)**, individual components of worsening renal function **(B)**, and renal-replacement therapy **(C)**. Each plot contains receiver operating characteristic for peak NGAL, first NGAL, and first creatinine, as well as AUC with 95% CIs. AUC = area under the curve; CI = confidence interval; NGAL = neutrophil gelatinase-associated lipocalin.

SUBGROUP ANALYSIS. Subjects were first stratified by eGFR greater or less than 60 ml/min/1.73 m^2. Subsequently, they were stratified at 2 cutpoints of the first NGAL: NGAL greater or less than 150 ng/dl, and greater or less than 109.3 ng/dl, the latter being the 80% sensitivity cutpoint for the composite secondary outcome. The percent of subjects who had any adverse outcome, by the different subgroups and NGAL cutpoints, is shown in **Figure 4A**.

The occurrence of adverse events increased with increasing NGAL and decreasing eGFR. Subjects with eGFR ≥60 ml/min/1.73 m^2 and NGAL less than the cutpoint had the fewest adverse outcomes, followed by those with eGFR <60 ml/min/1.73 m^2 and NGAL less than the cutpoint, then eGFR ≥60 ml/min/1.73 m^2 and NGAL greater than the cutpoint, and, lastly, those with eGFR <60 ml/min/1.73 m^2 and NGAL greater than the cutpoint, who had a marked increase in adverse outcomes (**Figures 4B and 4C**). In subjects with an eGFR <60 ml/min/1.73 m^2 and NGAL less than the cutpoint, a cutpoint of 150 ng/dl had 84.5% sensitivity and a 91.0% negative predictive value for excluding

FIGURE 3 Outcomes for In-Hospital Adverse Events



(A) Kaplan-Meier curve for the combined secondary outcome with subjects at risk is at the **bottom**. Approximately 50% of subjects at risk for an adverse event experienced an event during hospitalization. **(B)** Peak NGAL values (log-transformed) for the individual components of the combined secondary outcome are shown. **(C)** Receiver operating characteristic curves for the combined secondary outcome with peak NGAL, first NGAL, and first creatinine AUC and 95% CI. ino = inotrope/vasopressor; Neph Con = nephrology consultation; RRT = renal-replacement therapy; Vent = ventilator; WRF = worsening renal function; other abbreviations as in Figure 2.

the development of an adverse outcome, whereas a cutpoint of 109.3 ng/dl had a 90.3% sensitivity and 91.1% negative predictive value (Figures 4B and 4C).

DISCUSSION

WRF during hospitalization for AHF is associated with significant morbidity and mortality (2-4,6). Both mechanistic and iatrogenic perturbations are causative, including diuretic therapy, with its neurohormonal activation and maladaptive effects on renal function (18,19). Although serum creatinine is considered the standard functional biomarker for diagnosing WRF, it often does not increase until 1 to 3 days after injury has occurred, delaying diagnostic assessment and potential management. Thus, there is a clear unmet need for biomarkers to diagnose WRF earlier during the treatment of AHF, to potentially prevent or ameliorate progressive kidney damage and dysfunction.

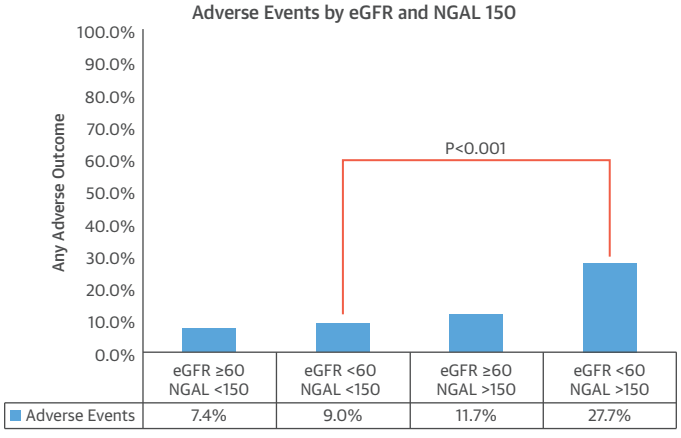
AKINESIS is one of the largest multicenter international trials exclusively evaluating the role of biomarkers for predicting the development of WRF or renal dysfunction requiring RRT in patients presenting with AHF treated with diuretic agents. More specifically, in this report, we examined a commercially available biomarker, plasma NGAL. Although analysis demonstrated a potential role for plasma NGAL in

FIGURE 4 Subgroup Analysis By Admission eGFR and First NGAL for In-Hospital Adverse Outcomes

A

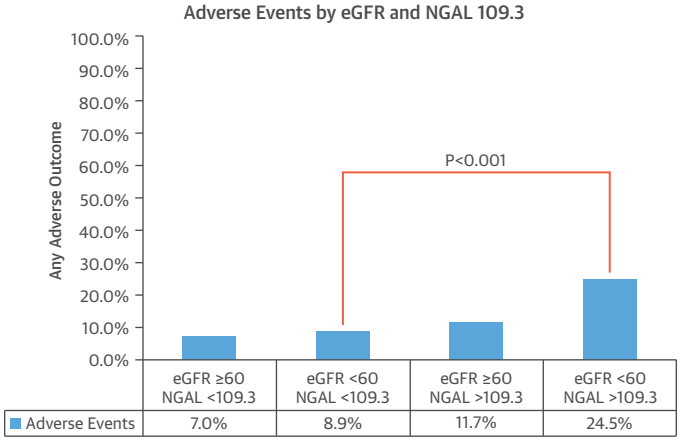
	GFR≥60 (n=414)		GFR<60 (n=492)	
NGAL (ng/ml)	<150 (n=320)	>150 (n=94)	<150 (n=178)	>150 (n=314)
Any Adverse Outcome	7.4%	11.7%	9.0%	27.7%
None	92.6%	88.3%	91.0%	72.3%
NGAL (ng/ml)	<109.3 (n=243)	>109.3 (n=171)	<109.3 (n=112)	>109.3 (n=380)
Any Adverse Outcome	7.0%	11.7%	8.9%	24.5%
None	93.0%	88.3%	91.1%	75.5%

B



Predictive Performance eGFR<60 NGAL<150 mg/mL		
Sensitivity	84.5%	(CI 76.0%-90.5%)
Specificity	41.7%	(CI 36.8%-46.7%)
Positive Predictive Value	27.7%	(CI 23.0%-33.0%)
Negative Predictive Value	91.0%	(CI 85.8%-94.5%)

C



Predictive Performance eGFR<60 NGAL<109.3 mg/mL		
Sensitivity	90.3%	(CI 82.8%-94.9%)
Specificity	26.2%	(CI 22.1%-30.9%)
Positive Predictive Value	24.5%	(CI 20.4%-29.1%)
Negative Predictive Value	91.1%	(CI 84.2%-95.3%)

(A) Percent of subjects with an adverse event stratified by eGFR and the 2 different NGAL cutpoints. Incidence of adverse events by subgroups of eGFR **(B)** and NGAL **(C)**, presented by increasing prevalence of events, with their respective sensitivities, specificities, positive predictive values, and negative predictive values displayed beside them. eGFR = estimated glomerular filtration rate; GFR = glomerular filtration rate; other abbreviations as in Figure 2.

predicting adverse outcomes, this was driven by nephrology consultation, and NGAL could not predict the development of WRF better than creatinine. There are several possible reasons why NGAL was not significant.

First, WRF may be caused by multiple factors, including AHF therapies; concurrent illness; or cardiorenal syndrome, a complex state consisting of multiple pathophysiologic processes (5,20). AKINESIS was designed to assess early development of WRF with diuretic therapy; however, we could not adequately assess other possible etiologies of WRF, or if WRF developed later during the hospital course or before presentation. Other, unaccounted factors may have contributed to the rise in NGAL or the development of WRF. NGAL has been associated with cardiovascular disease and may be a marker of disease severity (21). This association with disease severity may be reflected in our clinical composite secondary outcome.

Second, the definition and understanding of what constitutes AKI is changing (22,23). Renal injury can occur to varying degrees at different sites of the kidney and by multiple different mechanisms. WRF may be manifested as renal damage, functional change (classically termed pre-renal AKI), or both (classically termed acute tubular necrosis), with biomarkers of kidney damage or dysfunction detecting different types of renal injury (24). It seems that NGAL may not detect renal injury reflected by an increase in creatinine imposed by diuretic therapy (7). In a prior small study, NGAL did not change with the withdrawal and reinstitution of diuretic therapy (25).

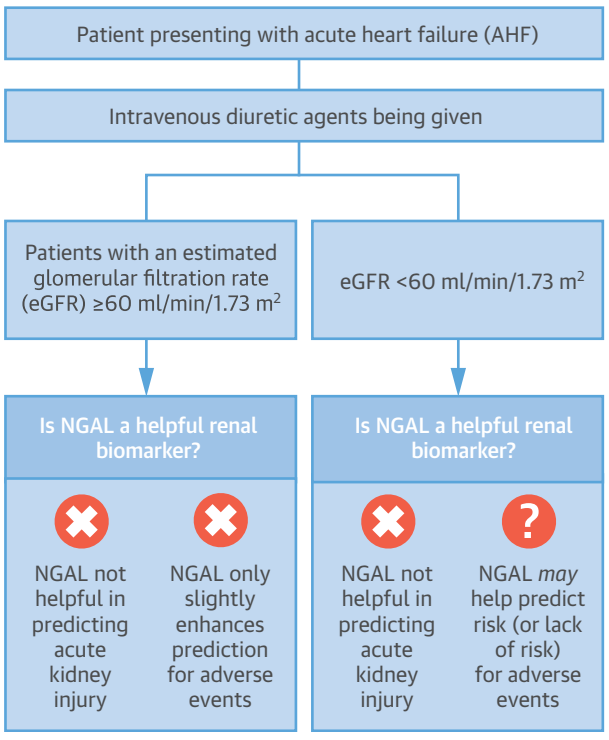
Third, the rate of WRF in AKINESIS was low, at 7.1%, compared with prior studies of NGAL, which have reported rates of 11.8% to 33.8%; however, a more liberal definition of a creatinine increase ≥0.3 mg/dl was used to define WRF in these studies (15,16). The low rate of WRF likely stems from our more stringent definition of WRF. Using an increase of ≥0.3 mg/dl as a definition, 30.6% patients developed WRF in our cohort. Even with this definition, the predictive value of NGAL was low. Notably, NGAL's predictive ability improved with increasing severity of kidney injury, as previously reported (26).

NGAL did have fair prognostic ability for adverse in-hospital outcomes (AUC: 0.691), although this was similar to creatinine (AUC: 0.686), and was driven by nephrology consultation, with a decrease in AUC with removal of nephrology consultation (AUC: 0.645). Previous studies have shown NGAL's association with adverse events (27–29). In the GALLANT study, plasma NGAL at discharge was shown to predict 30-day readmission for AHF and all-cause mortality, with an AUC of 0.73, which is similar to the AUC in AKINESIS (27). Furthermore, when grouping patients by eGFR and first NGAL values, we saw a stepwise increase in adverse events, as previously reported (28,30).

An exploratory finding was the low rate of adverse outcomes in subjects with an eGFR <60 ml/min/1.73 m^2 and NGAL <150 ng/dl, suggesting a possible role for NGAL (**Central Illustration**). However, this is a post hoc subgroup analysis, and is only hypothesis-generating. Further study is needed to see if this is a specific population for which NGAL can risk stratify.

Our study has multiple strengths. It is one of the largest prospective cohorts specifically studying

CENTRAL ILLUSTRATION Potential Diagnostic Algorithm for Neutrophil Gelatinase-Associated Lipocalin for Acute Kidney Injury



Maisel, A.S. et al. J Am Coll Cardiol. 2016;68(13):1420–31.

In patients presenting with AHF treated with intravenous diuretic agents, the results of AKINESIS do not support the use of NGAL in patients with an eGFR ≥60 ml/min/1.73 m^2. However, in patients with an eGFR <60 ml/min/1.73 m^2, NGAL may identify patients at low risk for adverse events. The proposed algorithm is the result of subgroup analysis and requires further study for confirmation. AHF = acute heart failure; AKINESIS = Acute Kidney Injury N-gal Evaluation of Symptomatic heart failure Study; eGFR = estimated glomerular filtration rate; NGAL = neutrophil gelatinase-associated lipocalin.

biomarkers of WRF in AHF. It suggests that to fully use tubular biomarkers to predict WRF and its complications in AHF, we might need to consider more robust definitions of WRF, including larger changes in creatinine over longer periods of time before improvement occurs, and the association with extraneous factors, such as continued use of angiotensin-converting enzyme inhibitors or other superimposed inflammatory conditions. Our data support the notion of Damman and Testani (7) that much of the creatinine elevation in AHF may be termed pseudo-WRF.

STUDY LIMITATIONS. Samples were collected after diuretic therapy was administered, in part, to not delay treatment, but this may have influenced NGAL levels. Samples were only collected early during the hospitalization, because the intent was to assess the development of early WRF. The first measured creatinine value was used as the baseline for assessment of WRF; however, this value may have already been elevated from a patient's baseline creatinine. Patients may have been experiencing WRF on admission not secondary to IV diuretic agents. In addition, creatinine measurements were not performed at a core laboratory and are subject to between-laboratory variability.

For outcomes, different definitions of WRF and adverse outcomes could have been used; however, analysis with alternative WRF definitions did not improve NGAL diagnostic utility. Our endpoints were on the basis of published reports at the time of inception of AKINESIS, and additional exploratory analyses have been prompted by recent changes in the understanding of WRF (22–24). Although in-hospital outcomes were closely monitored, events were not centrally validated. No interaction was found between NGAL and region; however, unmeasured differences in delivery of care between the United States and Europe still could have influenced results. Lastly, we lack post-discharge outcomes in this analysis, which could have implications for the utility of NGAL.

CONCLUSIONS

AKINESIS is a novel, large, multicenter cohort study exploring new biomarkers for AKI in AHF. AKINESIS does not support the routine use of NGAL for earlier detection of WRF or need for RRT in patients admitted with AHF and treated with diuretic agents. Also, NGAL overall was no more predictive of adverse outcomes than creatinine. There was a signal that a low NGAL value in patients with an eGFR <60 ml/min/1.73 m^2 was sensitive for predicting a low risk of adverse outcomes; however, this is only hypothesis-generating.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Deterioration of renal function has prognostic importance in patients with AHF, but a rise in the serum creatinine level is neither timely nor specific. Plasma levels of NGAL increase in acute kidney injury, and are associated with adverse outcomes in patients with chronic HF, but were no better than creatinine levels in predicting WRF from diuretic therapy or adverse in-hospital events in patients with AHF.

TRANSLATIONAL OUTLOOK: Further studies are needed to evaluate the value of biomarkers other than NGAL for predicting worsening renal function during diuretic therapy of patients with AHF.

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